REMARKS

The specification has been amended to place the application in conformance with standard United States Patent practice.

A substituted specification has been submitted with proper margins. Figs. 2 and 4 have been labeled. A marked-up version of the substitute specification, showing the margin changes, accompanies the substitute specification, as called for in 37 C.F.R. § 1.125.

Examination and allowance of claims 13-21 are respectfully requested.

Respectfully submitted,

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MARKED UP AMENDED SPECIFICATION PARAGRAPHS

Page 35, last paragraph that continues onto page 36

The disposition rate of staphylokinase-related antigen from blood following bolus injection of 100 μg/kg of the selected SakSTAR variants in groups of 4 hamsters could adequately be described by a sum of two exponential terms by graphical curve peeling (results not shown), from which the pharmacokinetic parameters t1/2α and Clp, summarized in Table 13 were derived. The pharmacokinetic parameters of dimeric SakSTAR(K109C) and SakSTAR(K102C-PEG) were markedly different from those of wild type SakSTAR. Initial plasma half-lives (t1/2(α)) were 3.6 and [....] 3.0 min and plasma clearances (Clp) were 0.52 and [.....] 0.32 mL/min, for dimeric SakSTAR(K109C) and SakSTAR(K102C-PEG), respectively. These results may be due to the increase of the Stokes radius of SakSTAR as a result of the dimerization or crosslinking with PEG. According to [size-eclusion] size-exclusion chromatography on Superdex50 by HPLC, dimeric SakSTAR(K109C) and SakSTAR(K102C-PEG) have apparent molecular weights of 33 kDa and 40 kDa, respectively.

Page 37, second *complete* paragraph

The present invention was initiated by the observation that certain "clustered charge-to-alanine" substitution variants of recombinant staphylokinase (SakSTAR variant (9)) had a reduced reactivity with antibodies induced by treatment with wild type SakSTAR (3,4) and induced less antibodies than wild type SakSTAR in patients with peripheral arterial occlusion (22,32,35). In an effort to optimize the specific activity versus antigenicity ratio, a comprehensive mutagenesis study, comprising the construction and expression of over 250 plasmids encoding SakSTAR variants, and the purification of the translation products was undertaken. The SakSTAR variants were characterized in terms of specific activity, affinity

towards a panel of murine monoclonal antibodies and absorption of SakSTAR specific antibodies from pooled plasma of 10 patients treated with wild type SakSTAR and of two subpools of 3 patients each which reacted strongly (subpool B) or poorly (subpool C) with the immunodominant epitope K74,E75,R77. In a later phase, an additional pool of 40 patients treated with wild-type SakSTAR was also used for absorption studies. The values obtained with both pools were in good agreement. Linear regression analysis yielded: [(Pool 10)=(Pool 40) + ..., with r=.....] (Pool 40) = 0.84 x (Pool 10) + 14, with r=0.94 and n=61.

Page 40, current paragraph

Intra-arterial administration of wild-type SakSTAR, SakSTAR(K74Q,E80A,D82A,K130T,K135R) or SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R) as a bolus of 2 mg followed by an infusion of 1 mg/hr in 6 patients with angiographically documented occlusion of a peripheral artery or bypass graft each, resulted in complete recanalization in 10 patients and partial recanalization in 2, without measurable systemic plasminogen activation. Following administration of wild-type or variant SakSTAR, neutralizing antibody titers and specific IgG levels remained low for one week. From the second or third week onwards, an increase of SakSTAR-neutralizing activity to ≥ 5 μg/mL plasma was observed in the 3 of the 6 patients given SakSTAR(K74Q,E80A,D82A,K130T,K135R), and in only [...] 1 of the [...] 6 patients given SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R). This immunization rate [of ....%] with the variants is significantly lower than the immunization rate of 80% observed in 70 patients treated with SakSTAR [(p=.... by Fisher's exact text)] (p=0.01 by 2 x 3 Chi square analysis). The antibodies induced by treatment with the SakSTAR variants were completely absorbed by SakSTAR, and by the respective variants in all but one patient with measurable neutralizing antibody levels, indicating that immunization was not due to neoepitopes generated by substitution but to residual epitopes in the SakSTAR template.

Page 67, fifth complete paragraph

Fig. 5. Time course of neutralizing activities (left panel) and specific IgG against administered agent (right panel) following intra-arterial infusion of SakSTAR [(circles, n=....)] (circles, n=15), SakSTAR(K74Q,E80A,D82A,K130T,K135R) (squares, n=6) or SakSTAR(E65D,K74R,E80A,D82A,K130T,K135R) (triangles, n=6) in patients with peripheral arterial occlusion. The data represent median values and 15-85 percentile ranges, in μg/mL.